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Cell hydration and proteolysis control in liver

Previous work from our laboratory established that cell hydration is one major determinant of proteolysis control in the intact liver [1], with potential relevance for the understanding of protein catabolism in health and disease [2]. This was confirmed by Meijer *et al.* [3] in a study on isolated rat hepatocytes. However, those authors claimed that cell swelling merely increases the sensitivity of proteolysis to amino acid inhibition, but is by itself ineffective. This conclusion was based on the finding that hypo-osmotic exposure of freshly isolated hepatocytes had no effect on proteolysis, but inhibited proteolysis when amino acids were added. These findings were in sharp contrast with the situation in the intact perfused liver: here, no amino acid supplementation is required for the strong anti-proteolytic effect of hypo-osmotic cell swelling. In his letter [4], Dr. Meijer now extrapolates his idea on an amino acid requirement for cell volume effects on proteolysis to the intact organ, by claiming that the small amount of amino acids generated in the perfused liver may be sufficient to mediate the hypotonicity effect on proteolysis. Although amino acids are inevitably present in proteolytic environments, this speculation is not very convincing. Compared with the single-pass liver perfusion system (which allows continuous wash-out of endogenously derived amino acids), amino acids are expected to accumulate in the closed experimental system of isolated hepatocytes to much higher values over a 1–2 h incubation period. Thus, the hypotonicity effects on proteolysis should even be pronounced in isolated hepatocytes, if Dr. Meijer's suggestion is correct [4]. However, hypo-osmolarity alone has no effect on proteolysis in isolated hepatocytes, but strongly inhibits in perfused liver. In perfused liver, disruption of microtubules by colchicine almost completely abolishes the anti-proteolytic effect of cell swelling, but does not affect inhibition of proteolysis by phenylalanine or asparagine, *i.e.* amino acids which affect proteolysis largely independent of cell volume changes [5]. Such a difference in colchicine sensitivity is difficult to reconcile with the view that cell swelling in perfused liver merely increases the sensitivity of proteolysis to amino acids. It rather suggests that involvement of different mechanisms mediating the anti-proteolytic effect of asparagine/phenylalanine on the one hand and cell swelling on the other. Further, previous liver-perfusion studies have shown that the anti-proteolytic effect of glutamine and glycine is quantitatively mimicked by hypo-

osmotic cell swelling to an extent as these amino acids do ([1] and references quoted therein). If cell swelling would merely enhance the amino acid action on proteolysis, one would predict a stronger inhibition of proteolysis when the same degree of cell swelling is induced by glutamine/glycine instead by hypo-osmolarity. This, however, is not observed. In addition, whereas hyperosmotic exposure can stimulate proteolysis in perfused liver [1], it surprisingly inhibits proteolysis in isolated hepatocytes, even in the absence of amino acids [3]. Thus there is enough experimental evidence for profound differences regarding cell volume control of proteolysis between the hepatocyte *in situ* and the hepatocyte in suspension.

These differences apparently also hold for insulin effects on proteolysis. Depending on the experimental system used, inhibition of hepatic proteolysis by insulin ranges between 0 and 23% or even more [1,5,6], with little or no effect in isolated hepatocytes [5,6], but a strong inhibitory effect in the perfused liver ([1,5] and references therein). We do not agree with Dr. Meijer that such differences are 'quite minor' [4]. As with hypo-osmolarity, Dr. Meijer suggests that insulin potentiates the sensitivity of autophagic proteolysis to inhibition by low concentrations of amino acids, whereas in their absence insulin has little effect [4]. It is the same rationale as above which makes it difficult to explain the strong anti-proteolytic effect of insulin and its colchicine-sensitivity in perfused liver [1,5] on the basis of a potentiation of amino acid action. Alternative explanations must be considered. One possibility was addressed in [5] by the demonstration that cultivation of hepatocytes (derived from starved rats) increased significantly the anti-proteolytic effect of insulin from virtually zero to 14% and the microtubule-dependence of the insulin effect on proteolysis. The nutritional state also requires attention. Hepatic insulin resistance during fasting is long known and is, despite an increased number of insulin receptors [7], associated with a decreased insulin-receptor tyrosine kinase activity [8]. Indeed, insulin-induced K^+ uptake and cell swelling, as well as its anti-proteolytic potency are diminished by 60–70% when livers from starved rats are used for perfusion [8a]. Many studies on isolated hepatocytes used 24 h-starved animals (*e.g.* [3,5,6]), and small insulin effects on proteolysis are therefore not surprising. It is not known whether addition of amino acids restores insulin-responsiveness of isolated 'starved' hepatocytes. But in such a case, the observations by Meijer and co-workers [6], namely that insulin effects can be observed in isolated hepatocytes only on addition of amino acids, may reflect just restoration of insulin sensitivity, but, as claimed in [4], a potentiation of the sensitivity of proteolysis to amino acids. Likewise, cell swelling should not necessarily increase the sensitivity of proteolysis to amino acid inhibition, as stated in [3,4]. An alternative explanation could be that amino acids are required to restore the sensitivity of proteolysis to cell volume changes in isolated hepatocytes, but not in perfused liver.

The situation becomes even more confusing when one considers that proteolysis was studied in [3] in the presence of cycloheximide in order to block protein synthesis. Although it is stated in [3] that cycloheximide has no effect on proteolysis, this compound by itself interferes with proteolysis in perfused liver [5] and in hepatocyte monolayers [9], and in the latter system cycloheximide abolished the insulin effects.

The differences regarding published data on proteolysis control by cell volume, insulin and amino acids in the hepatocyte *in situ* or in suspension are not surprising when one considers the loss of hepatocyte polarity, cytoskeletal derangements and loss of cell-cell contacts accompanying hepatocyte isolation. Evidence for a crucial role of intact microtubules for the cell volume and insulin control of proteolysis and some other volume-sensitive

pathways has been given [5,10], and the sensitivity of proteolysis to insulin and hypo-osmolarity is restored in part upon cultivation of hepatocytes [5]. Although this does not imply that the intact organ perfusion system is 'more physiological' than an isolated hepatocyte suspension (both systems are more or less unphysiological model systems), some regulatory properties may be lost or change upon cell isolation. It is important to acknowledge such limitations.

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